Research Article

www.ijrap.net (ISSN:2229–3566)

STANDARDIZATION OF A SIDDHA HERBOMINERAL FORMULATION: KARASOODA SATHU PARPAM

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Received on: 19/06/20 Accepted on: 04/08/20

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DOI: 10.7897/2277-4343.1105150

ABSTRACT

Siddha system is one of the ancient medical systems in India. The siddha is capable of treating all types of disease other than emergency cases. Karasooda Sathu Parpam is a traditional siddha herbomineral drug. The aim of the present study is to investigate the phytochemical, physiochemical and TLC, HPTLC of a traditional Shastric siddha herbomineral formulation known as Karasooda Sathu Parpam. Preliminary phytochemical analysis, HPTLC analysis and physicochemical parameters such as ash values, extractive values and loss of drying were determined as per Pharmacopoeial Laboratory for Indian Medicines (PLIM) guidelines. Phytochemical analysis of different extracts gave positive test for Alkaloids, Flavonoids, Glycosides, Steroids, Triterpenoids, Coumarin, Phenol, Tannins, Saponin, Proteins, Anthocyanin and carbohydrates. HPTLC fingerprint analysis of extracts showed the presence of possible number of components. Physicochemical parameters such as the total ash value was found to be 9.43 ± 0.19 %, acid insoluble ash value is 0.5133 ± 0.117% and loss of drying at 105ºc is 0.68 ± 0.10%. The water-soluble extractives and alcohol soluble extractives were found to be 0.4667 ± 0.22% and 0.2567 ± 0.086%. Biochemical analysis revealed the presence of Sulphate, Chloride, Carbonate, Iron, Zinc, Calcium, Magnesium, Ammonium, Starch, Alkaloids. Qualitative analysis revealed the presence of Sulphate, Chloride, Carbonate, Aluminum, Iron, Zinc, Calcium, Magnesium, Ammonium, Starch and alkaloids. The study highlights the appropriate application of modern scientific methods for developing the new insights into metal-based Siddha drugs.

Keywords: Karasooda Sathu Parpam, Standardization, Siddha, Herbomineral drug.

INTRODUCTION

Siddha system of medicine is followed in southern part of India especially Tamil Nadu. It is named as Tamil medicine.[1] This system was formulated and established by the eminent Siddhars about more than millennium back and hence it was named as siddha medicine. In siddha system, Medicine (Marundhu) is classified into two major types. They are Internal medicine (Aga Marundhu) and external medicine (Pura Marundhu). There are thirty-two internal medicine and thirty-two external medicines. Siddha medicine includes the herbal products, inorganic substances and animal products that lead to different formulations ranging from plant decoctions to herbomineral drugs like Parpam, chenduram, Chunnam, etc. According to siddha doctrines, everything found in nature has two qualities; good and bad. Numerous methods of purification for every single metal and mineral were employed by the siddha system of medicine. There by it ensures the safety of the Medicine.[2] Parpams are the powder substances generally obtained by calcification of purified metals, minerals and animal products by specific process. They are calcined in closed crucibles in pits and with cow dung cakes for the process of Puddam. Generally these methods of preparation of Siddha medicine involves conversation of minerals or metals into oxide or sulphide form by various herbal treatment followed by repeated high temperature calcinations and grinding cycles.[3] Parpam thus obtained constitute ultra-small particles and are taken along with vehicles such as milk, ghee, butter, honey etc according to disease. This makes these drugs easily assailable and thereby eliminates their harmful effects and enhances their biocompatibility. Standardization is the process of implementing and developing technical standards. Standardization helps to maximize the compatibility, interoperability, safety, repeatability or quality. It can facilitate commoditization of formerly custom process. The present study investigate the physicochemical properties of traditional Indian Siddha preparation Karasooda Sathu Parpam which is mentioned in the Siddha text Sikicha Rathina Deepam which can be widely used for treating Kalladaippu, Neeradaippu and Sathaiadaippu and other urinary disorders.[4]

MATERIAL AND METHODS

Preparation of Karasooda Sathu Parpam (Method I)

Karasooda Sathu Parpam was prepared as per the methodology mentioned in the Shastric Siddha text recognized by drugs and cosmetic act 1940. The raw drugs were procured from various well reputed raw drug stores in Chennai, Tamil Nadu, India and mineral drugs were authenticated by, Associate professor/Department of Gunapadam, National Institute of Siddha, Chennai. Each drug was purified by the purification methods mentioned in followed by Siddha texts. The Parpam was prepared by grinding the raw materials with lemon juice, Puddam method. They are usually prescribed in the dose 488 mg/day (Panavedai/twice a day) and recommended to be taken with honey.[5]

Ingredients

Purified Venkaram – 1 Palam (35 gm)
Purified Silasathu - 1 Palam (35 gm)
Lemon juice – Required qty.[2,6]

Purification of ingredients of the trial drug

Purification of Vengaram

1 Palam (35 gm) of Vengaram was fried till the moisture gets removed.

Purification of Karpura Silasathu

1 Palam (35 gm) of Silasathu was powdered and soaked in 2 part of tender coconut water for 24 hours. It was then filtered and dried. This process was repeated 2 times.[8]

Physicochemical analysis

Figure 1: Sample Description[9,10]

Table 1: Sample Description

State	Solid
Nature	Very fine
Odour	Very mild
Touch	Soft – Moderately coarse
Flow Property	Free
Appearance	Greyish white

Method of trial drug preparation

35 gm of Vengaram and 35gm Karpura Silasathu were purified separately and its combination has been grinded with lemon juice for 6 hrs and then made into tablets (Villai). These tablets were dried in sunlight and then put into mud lid and surmounted an equivalent mud lid and sealed by clay plaster winded it for 2 times and then burnt using Varrati (dried cow dung) and allowed it to cool. Finally, the mud lid was taken out and seal was opened to collect the leftover available in the lid. This leftover was grinded as fine powder. This powder Karasooda Sathu Parpam was stored in closed container.[7]

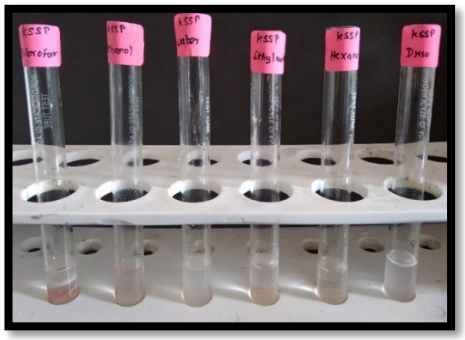


Figure 2: Solubility Profile

Table 2: Solubility Profile

S. No.	Solvent Used	Solubility / Dispersibility
1	Chloroform	Insoluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl acetate	Insoluble
5	Hexane	Insoluble
6	DMSO	Soluble

Table 3: Confirmatory Specification for Parpam

Parameter	Observation for MP
Fineness	Confirms the standard for fineness as per the particle size analysis and flow property of the sample
Float on Water	Confirms the test
Smokeless	Confirms the test
Taste less	Confirms the property
Luster less	Confirms the property

Method for physicochemical analysis

Percentage Loss on Drying

Test drug was accurately weighed in evaporating dish. The sample was dried at 105ºC for 5 hours and then weighed.

Determination of Total Ash

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 ºC until it turns white in color which indicates absence of carbon. Percentage of total ash was calculated with reference to the weight of air-dried drug.

Determination of Acid Insoluble Ash

The ash obtained by total ash test was boiled with 25 ml of dilute hydrochloric acid for 6 minutes. Then the insoluble matter is collected in crucible and washed with hot water and ignited to constant weight. Percentage of acid insoluble ash was calculated with reference to the weight of air-dried ash.

Determination of Alcohol Soluble Extractive

Test sample was macerated with 100 ml of ethanol in a closed flask for twenty-four hours, shaking frequently for six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish and dry at 105ºC,

to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Determination of Water-Soluble Extractive

Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently for six hours and allowing it to stand and for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish and dry at 105ºC, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

Result of physicochemical analysis

Physicochemical analysis revealed the total ash value was found to be 9.43 ± 0.19 %, acid insoluble ash value is 0.5133 ± 0.117 %, and loss of drying at 105ºc is 0.68 ± 0.10 %. The water-soluble extractives and alcohol soluble extractives were found to be 0.4667 ± 0.22 % and 0.2567 ± 0.086 %.

Table 4: Physicochemical evaluation of Karasooda Sathu Parpam

S. No.	Parameter	Mean (n = 3) SD
1.	Loss on Drying at 105 °C (%)	0.68 ± 0.10
2.	Total Ash (%)	9.43 ± 0.19
3.	Acid insoluble Ash (%)	0.5133 ± 0.117
4.	Water soluble Extractive (%)	0.4667 ± 0.22
5.	Alcohol Soluble Extractive (%)	0.2567 ± 0.086

Biochemical analysis

Table 5: Method for biochemical analysis

S. No.	Experiment	Observation	Inference
I. Test for Acid Radicals			
1.	Test for Sulphate: 2 ml of the above prepared extract is taken in a test tube to this added 2 ml of 4% ammonium oxalate solution.	Cloudy appearance present	Presence of Sulphate
2.	Test for Chloride: 2 ml of the above prepared extract is added with 2 ml of dil-HNO_3 till the effervescence ceases. Then 2 ml of silver nitrate solution is added.	Cloudy appearance Present	Presence of Chloride
3.	Test for Phosphate: 2 ml of the extract is treated with 2 ml of ammonium molybdate solution and 2 ml of con. HNO_3	No yellow colour Appearance	Absence of Phosphate
4.	Test for Carbonate: 2 ml of the extract is treated with 2 ml magnesium sulphate solution	Cloudy appearance Present	Presence of Carbonate
5.	Test for Nitrate: 1 gm of the substance is heated with copper turning and concentrated H_2So4 and viewed the test tube vertically down	No Brown gas is Evolved	Absence of Nitrate
6.	Test for Sulphide: 1 gm of the substance is treated with 2 ml of con. HCLs	No Rotten Egg Smelling gas evolved	Absence of Sulphide
II. Test for Basic Radicals			
7.	Test for Lead: 2 ml of the extract is added with 2 ml of potassium iodine solution	No yellow precipitate is obtained.	Absence of Lead
8.	Test for Aluminium: To the 2 ml of extract sodium hydroxide is added in drops to excess.	No characteristic changes.	Absence of Aluminium
9.	Test for Iron: To the 2 ml of extract add 2ml of ammonium Thiocynate solution	Mild red colour appear	Presence of Iron
10.	Test for Zinc: To 2 ml of the extract sodium hydroxide solution is added in drops to excess	White precipitate is formed	Presence of Zinc
11.	Test for Calcium: 2 ml of the extract is added with 2 ml of 4% ammonium oxalate Solution	Cloudy appearance and white precipitate is obtained	Presence of Calcium
12.	Test for Magnesium: To 2 ml of extract sodium hydroxide solution is added in drops to excess.	White precipitate is not obtained	Presence of Magnesium
13.	Test for Ammonium: To 2 ml of extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added.	Brown colour Appeared	Presence of Ammonium
14.	Test for Mercury: 2 ml of the extract is treated with 2 ml of sodium hydroxide solution	No yellow precipitate is obtained	Absence of Mercury
15.	Test for Arsenic: 2 ml of the extract is treated with 2 ml of sodium hydroxide solution	No brownish red precipitate is obtained	Absence of Arsenic

	III. Miscellaneous		
16.	Test for Starch: 2 ml of extract is treated with weak iodine solution	Blue colour Developed	Presence of Starch
17.	Test for Reducing Sugar: 5 ml of Benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again, boil it for 2 minutes. The Color changes are noted.	No Brick red colour Developed	Absence of Reducing sugar
18.	Test for The Alkaloids: a) 2 ml of the extract is treated with 2 ml of potassium iodide solution. b) 2 ml of the extract is treated with 2 ml of picric acid. c) 2 ml of the extract is treated with 2 ml of phosphotungstic acid.	Yellow colour Developed	Presence of Alkaloid
19.	Test for Tannic Acid: 2 ml of extract is treated with 2 ml of ferric chloride solution	Block precipitate is Obtained	Absence of Tannic Acid
20.	Test for Amino Acid: 2 drops of the extract is placed on a filter paper and dried well	No Violet colour Developed	Absence of Amino Acids
21.	Test for Type Of Compound: 2 ml of the extract is treated with 2 ml of ferric chloride solution	No Brown colour Developed No red colour developed No violet colour developed No Blue colour developed.	Absence of Oxy quinol, epinephrine and Pyro catechol Antipyrine, Aliphatic Amino acids and malonic acid are absent. Salicylate and resorcinol are absent. Morphine, Phenol cresol and hydroquinone are absent.[11]

Result of biochemical analysis

Biochemical analysis revealed the presence of Sulphate, Chloride, Carbonate, Iron, Zinc, Calcium, Magnesium, Ammonium, Starch, Alkaloids.

Table 6: Biochemical analysis of Karasooda Sathu Parpam - Test of acid radicals

Analytical test	Inference
Sulphate	Presence of Sulphate
Chloride	Presence of Chloride
Phosphate	Absence of Phosphate
Carbonate	Presence of Carbonate
Nitrate	Absence of Nitrate
Sulphide	Absence of Sulphide
Test for basic radicals	
Lead	Absence of Lead
Aluminium	Absence of Aluminium
Iron	Presence of Iron
Zinc	Presence of Zinc
Calcium	Presence of Calcium
Magnesium	Presence of Magnesium
Ammonium	Presence of Ammonium
Mercury	Absence of Mercury
Arsenic	Absence of Arsenic
Other constituents	
Starch	Presence of Starch
Reducing sugar	Absence of Reducing sugar
Alkaloids	Presence of Alkaloids
Tannic acid	Absence of Tannic acid
Amino acid	Absence of Amino acid
Compound	Absence of types os compound

Phytochemical analysis

Method for phytochemical analysis

Test for alkaloids

Mayer's Test: To the test sample, 2 ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for Coumarins

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of Coumarins is indicated by the formation of yellow color.

Test for saponins

To the test sample, 5 ml of water was added, and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

Test for tannins

To the test sample, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for glycosides- Bontrager's Test

Test drug is hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated, and 10% ammonia solution is added to it. Pink colour indicates presence of glycosides.

Test for flavonoids

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color indicates the presence of Flavonoids.

Test for phenols

Lead acetate test

To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids

To the test sample, 2 ml of chloroform was added with few drops of conc. Sulphuric acid (3 ml) and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Triterpenoids

Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of triterpenoids.

Test for Cyanins

Anthocyanin

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C. Formation of bluish green colour indicates the presence of anthocyanin.

Test for Carbohydrates - Benedict's test

To the test sample about 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Proteins (Biuret Test)

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.[12]

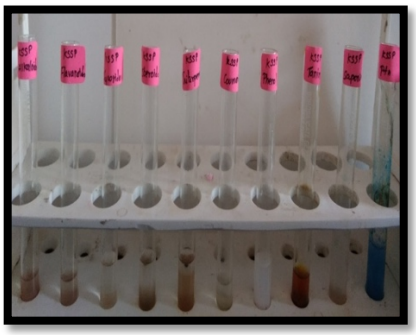


Figure 3: Qualitative Phytochemical Investigation

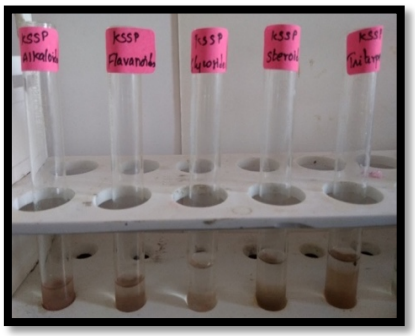


Figure 4: Test for Alkaloids, Flavonoids, Glycosides, Steroids and Triterpenoids

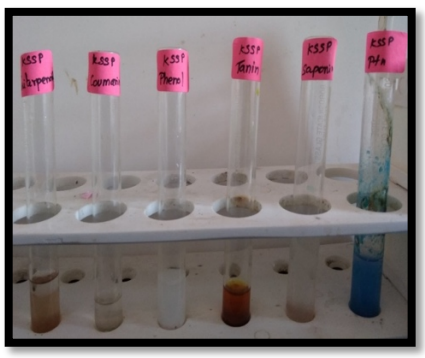


Figure 5: Test for Coumarin, Phenol, Tannins, Saponin, Proteins

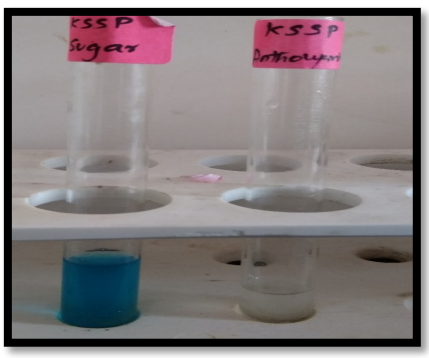


Figure 6: Anthocyanin and carbohydrates

Result of phytochemical analysis

Phytochemical analysis revealed the presence of Alkaloids, Flavonoids, Tannin, Protein, Saponins**.**

Table 7: Phytochemical analysis of Karasooda Sathu Parpam

S. No.	Test	Observation
1	Alkaloids	+
2	Flavonoids	+
3	Glycosides	-
4	Steroids	-
5	Triterpenoids	-
6	Coumarin	-
7	Phenol	-
8	Tannin	+
9	Protein	+
10	Saponins	+
11	Sugar	-
12	Anthocyanin	-
13	Betacyanin	-

Indicates Positive and - -> Indicates Negative+ ->

TLC analysis

Procedure of TLC analysis

TLC Analysis

Test sample was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel 60F254, 7 X 6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette was used to spot the sample for TLC applied sample volume 10-micro liter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with the specified solvent system after the run plates are dried and was observed using visible light Short-wave UV light 254 nm and light long-wave UV light 365 nm.[13]

HPTLC analysis

High Performance Thin Layer Chromatography analysis

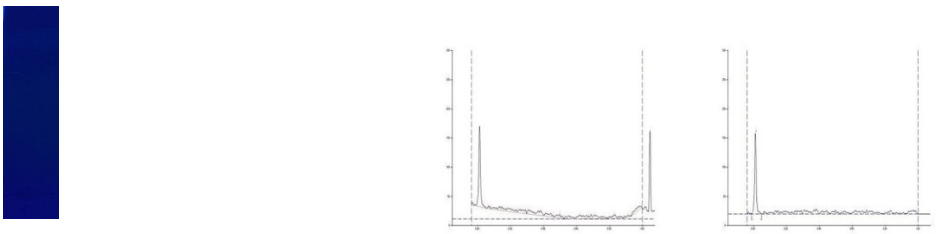
HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Thus, this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of Phyto therapeutics.[14]

Chromatogram Development

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 366 nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic fingerprint was developed for the detection of phytoconstituents present in each sample and their respective Rf values were tabulated.



HPTLC finger printing of Sample KSSP

Peak Table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.01	1.0	0.01	138.3	100.00	0.05	0.1	1090.6	100.00

TLC Visualization of KSSP - TLC plate visualization at 366 nm

Report

HPTLC finger printing analysis of the sample reveals the presence of one peak. Rf value of the peak was found to be 0.01. Peak 1 occupies the major percentage of area of 100 which denotes the abundant existence of such compound.

DISCUSSION

The data obtained from the scanning through CAMAG software revealed the presence of phytoconstituents such as alkaloids, Flavonoids, Tannin, Protein and Saponin positive in the HPTLC analysis. HPTLC finger printing analysis of the sample reveals the presence of one peak. Rf value of the peak was found to be 0.01. Peak 1 occupies the major percentage of area of 100 which denotes the abundant existence of such compound. The phytochemical analysis confirmed the presence of alkaloids, Coumarins, Saponins, Tannins, Glycosides, Flavonoids and Phenols, steroids, Triterpenoids, protein and cyanine. The physiochemical analysis showed the insoluble chloroform, insoluble ethyl acetate and insoluble hexane. The loss on drying at 105ºc was only 0.68 % w/w; hence the drug will not lose much of its volume on exposure to this range of temperature. Biochemical analysis revealed the presence of Sulphate, Chloride, Carbonate, Iron, Zinc, Calcium, Magnesium, Ammonium, Starch, Alkaloids. Qualitative analysis revealed the presence of Sulphate, Chloride, Carbonate, Aluminum, Iron, Zinc, Calcium, Magnesium, Ammonium, Starch and alkaloids might be attributing therapeutic activity of the Parpam.

CONCLUSION

The above analysis of Karasooda Sathu Parpam showed the presence of phytochemicalsis confirmatory specification of Parpam. Karasooda Sathu Parpam confirmed the standard for fineness as per particle size analysis and flow property of the sample; considering Parpam nano particle which has the quickly absorbed and brings recovery. So, this Karasooda Sathu Parpam will have the new way to treat the renal calculi and quick recovery.

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Cite this article as:

P. Sharmila *et al*. Standardization of a Siddha Herbomineral Formulation: Karasooda Sathu Parpam. Int. J. Res. Ayurveda Pharm. 2020;11(5):103-109 http://dx.doi.org/10.7897/2277-4343.1105150

Source of support: Nil, Conflict of interest: None Declared